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Basal Cell Carcinoma-Like Epidermal Changes Overlying Dermatofibromas Often Reveal Loss of Heterozygosity in the PTCH Gene

To the Editor:

Dermatofibromas (DF) are benign dermal tumors that frequently exhibit a spectrum of overlying epidermal acanthosis. Mild acanthosis is the most common finding. Occasionally, significant basaloid proliferation is observed, and rarely, follicular and even basal cell carcinoma (BCC)-like changes may be seen. Whether the latter represent actual BCC or are instead a reactive hyperplasia, perhaps with differentiation towards follicular structures, has been disputed.

The *patch* (PTCH) gene is a developmental gene involved with spacial organization in the Drosophilia fruit fly and mice, and it is now known to also function as a tumor suppressor gene in human tumors. Germ-line mutations in PTCH have been detected in almost all cases of the nevoid basal cell carcinoma syndrome, and somatic mutations in PTCH are found in over one-third of all sporadic basal cell carcinomas (Gailani and Bale, 1997). It has been suggested that PTCH may even represent a "gatekeeper gene" for BCC development (Sidransky, 1996); however, while PTCH mutations were originally thought to be present exclusively in BCC, a recent study of trichoepitheliomas, which are benign follicular neoplasms that may bear some histologic similarities to BCC, found that two of nine specimens also contained mutations in PTCH (Vorechovsky *et al*, 1997).

We hypothesized that the follicular or BCC-like changes overlying DF might represent early clonal proliferations or even precursors to BCC. Based on Knudson's hypothesis, loss of heterozygosity (LOH) often represents the second step in tumor suppressor gene inactivation (Knudson, 1971). Because PTCH inactivation occurs frequently in BCC development, we analyzed five DF with follicular or BCC-like histologic changes (**Fig 1**) and five DF with mild acanthosis (data not shown) for evidence of LOH at the PTCH locus. The epithelial portion of each histologic specimen was carefully microdissected off of its slide using a fine needle under an inverted microscope. The tissue was then deparaffinized in xylene and the DNA was extracted (Nawroz *et al*, 1994). Normal and tumor DNA was analyzed for LOH after polymerase chain reaction amplification of polymorphic dinucleotide repeat sequences. We chose four markers at the PTCH locus for microsatellite analysis (**Fig 2*a***). These markers are highly polymorphic and one exists within the PTCH gene itself. Polymerase chain reaction products were separated by electrophoresis followed by autoradiography. For informative cases allelic loss was scored and confirmed by two independent observers (P.L. and D.S.). Loss was scored if the intensity of one allele was at least 30% reduced in the tumor compared with the normal DNA.

Polymerase chain reaction-based microsatellite analysis revealed LOH of the PTCH locus in three of the five specimens with follicular or BCC-like changes (**Fig 2*b***) and in none of the five specimens with mild acanthosis (data not shown). In each positive case, LOH was present in all informative markers, confirming loss of at least one PTCH allele in each of the samples. LOH clearly indicates the presence of a clonal process in the epidermis overlying these dermal tumors. Any contamination of sample tissues by normal cells would only have the effect of underrepresenting the presence of PTCH mutations in our samples, by producing false negative but not false positive results.

This evidence indicates clonality in what is historically described as a reactive hyperplastic process. Moreover, the presence of LOH at the PTCH locus in these BCC-like basaloid proliferations is consistent with, but not diagnostic of, PTCH inactivation and frank BCC. Because PTCH mutations and LOH at the PTCH locus have been found in another benign follicular epidermal neoplasm, trichoepithelioma (Vorechovsky *et al*, 1997), the presence of PTCH mutations in our specimens cannot differentiate between follicular or BCC-like changes and true BCC.

In summary, we have established the presence of a clonal proliferation, probably secondary to PTCH inactivation, in a majority of DF

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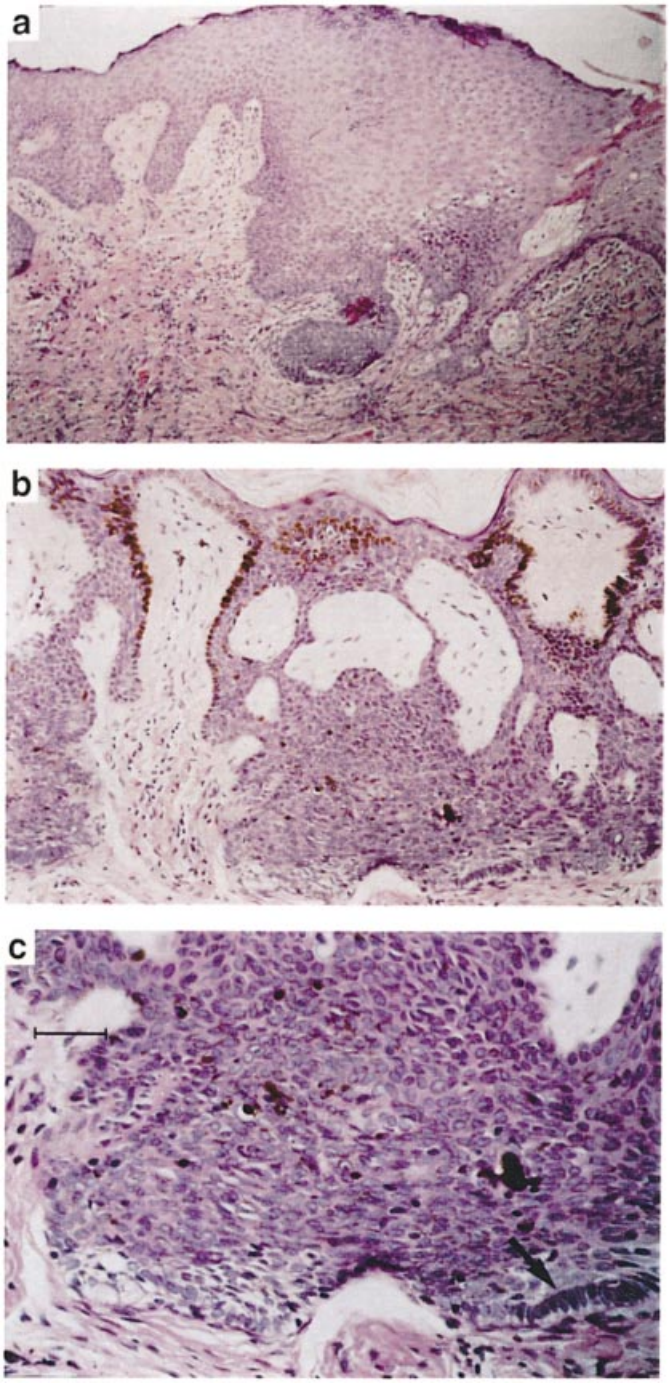

Figure 1. Epidermal proliferative changes overlying dermatofibromas. (*a*) Acanthosis with occasional immature follicles and sebaceous glands is commonly seen. (*b*) In rare cases, changes resemble basal cell carcinoma. (*c*) Higher magnification of (*b*) reveals crowding of the basal layer, peripheral palisading of basal epidermal nuclei (*arrow*), mitotic activity, and stromal retraction identical to that seen in basal cell carcinoma. *Scale bar*: (*a*, *b*) 104 μm; (*c*) 52 μm.

with extensive overlying epidermal basaloid proliferation. In the remaining two specimens, LOH may have been obscured by normal cell contamination, or PTCH inactivation may occur by small intragenic mutations of both PTCH alleles without LOH. Sequence

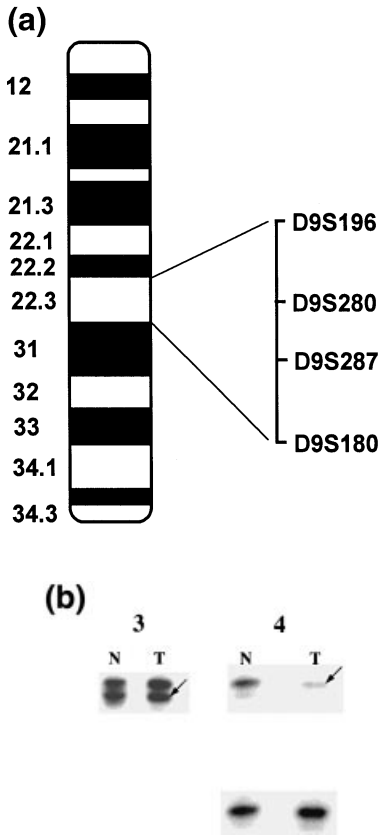

Figure 2. LOH on chromosomal arm 9q in DF. (*a*) Four microsatellite markers were used. (*b*) LOH analysis on chromosomal arm 9q for two cases of DF. Representative DF with BCC or follicle-like epidermal basaloid proliferation (T) and corresponding normal tissue (N) are shown for microsatellite marker D9S280. The lost allele is indicated by the *arrows*. Case 3 demonstrates loss of the bottom allele and case 4 of the top allele. See text for details.

analysis of the remaining allele remains to be done to formally demonstrate PTCH inactivation in these lesions. It appears likely that PTCH inactivation develops in a number of cutaneous neoplasms with basaloid proliferation in addition to BCC.

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